

CLAIMS

1. A modified Ca²⁺-binding polypeptide comprising
 - 5 a) a first chromophor of a donor-acceptor-pair for FRET (Fluorescence Resonance Energy Transfer),
 - b) a Ca²⁺-binding polypeptide with an identity of at least 80% to a 30 amino acid long polypeptide sequence of human troponin C or chicken skeletal muscle troponin C or drosophila troponin C isoform 1, and
 - 10 c) a second chromophor of a donor-acceptor-pair for FRET.
2. The polypeptide of claim 2, wherein the first chromophor is a fluorescent polypeptide capable of serving as a donor-chromophor in a donor-acceptor-pair for FRET and the second chromophor is a fluorescent polypeptide capable of serving as an acceptor-chromophor in a donor-acceptor-pair for FRET.
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3. The polypeptide of claim 2, wherein the modified polypeptide is a fusion polypeptide wherein the order of the three linked polypeptides starting from the N-terminus of the fusion polypeptide is a)-b)-c) or c)-b)-a).
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4. The polypeptide of any one of claims 1 to 3, wherein the first chromophor is selected from the group consisting of CFP, EGFP, YFP, DsFP 483, AmCyan, Azami-Green, Cop-Green and As499, particularly wherein the first chromophor is CFP.
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5. The polypeptide of any one of claims 1 to 4, wherein the second chromophor is selected from the group consisting of YFP, DsRed, zFP 538, HcRed, EqFP 611, Phi-Yellow and AsFP 595, particularly wherein the second chromophor is YFP.
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6. The polypeptide of any one of claims 1 to 5, wherein the Ca^{2+} -binding polypeptide comprises at least one Ca^{2+} -binding EF-hand.
- 5 7. The polypeptide of any one of claims 1 to 6, wherein the Ca^{2+} -binding polypeptide comprises a polypeptide sequence having at least 60% identity to amino acids 15 to 163 of chicken skeletal muscle troponin C or amino acids 1 to 161 of human cardiac troponin C or amino acids 5 to 154 of drosophila troponin C isoform 1.
- 10 8. The polypeptide of any one of claims 1 to 7, further comprising glycine-rich linker peptides N-terminal or C-terminal to polypeptide b).
9. The polypeptide of any one of claims 1 to 8, further comprising a localization signal, in particular a nuclear localization sequence, a nuclear export
15 sequence, an endoplasmic reticulum localization sequence, a peroxisome localization sequence, a mitochondrial import sequence, a mitochondrial localization sequence, a cell membrane targeting sequence, most preferably a cell membrane targeting sequence mediating localization to pre- or post-synaptic structures.
- 20 10. The polypeptide of any one of claims 1 to 9 which exhibits a ratio change upon Ca^{2+} -addition of more than 30%, preferably from 50% to 200%, more preferably from 80% to 180% and most preferably from 100% to
25 150%.
11. The polypeptide of any one of claims 1 to 10 which has a K_d for Ca^{2+} of from 50 nM to 400 μM , preferably of from 100 nM to 100 μM and most preferably of from 250 nM to 35 μM .

12. The polypeptide of claim 3 selected from any one of the polypeptides of SEQ ID NO. 2, 4, 6, 8, 10, 12, 14, 16, 18, 32, 34, or 42, preferably 2, 4, 34, or 42.
- 5 13. A nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide according to claims 3 to 12, preferably a nucleic acid sequence of SEQ ID NO 1, 3, 33, or 41.
- 10 14. An expression vector containing the nucleic acid molecule of claim 13, preferably further comprising expression control sequences operatively linked to a nucleic acid encoding a polypeptide according to claims 3 to 12.
- 15 15. A host cell, particularly a mammalian, non-human cell, inside or outside of the animal body or a human cell outside of the human body, comprising a polypeptide according to claims 3 to 12 and/or a nucleic acid according to claim 13 and/or an expression vector according to claim 14.
- 20 16. A transgenic animal comprising a polypeptide according to claims 3 to 12 and/or a nucleic acid according to claim 13 and/or an expression vector according to claim 14 and/or a host cell according to claim 15.
- 25 17. A method for the detection of changes in the local Ca^{2+} -concentration comprising the following steps:
- a) providing a cell or a subcellular membraneous fraction of a cell comprising a Ca^{2+} -binding polypeptide according to any one of claims 1 to 12; and
 - b) inducing a change in the local Ca^{2+} -concentration; and
 - c) measuring FRET between the donor and the acceptor chromophore of the donor-acceptor-pair of said polypeptide according to any one of
- 30 claims 1 to 12, which is indicative of the change in the local Ca^{2+} -concentration.

18. The method of claim 16, wherein the cell of step a) is a host cell according to claim 15.
19. The method of claim 16, wherein the subcellular membraneous fraction is
5 an organell, in particular a mitochondrium, a peroxisome or a nucleus, or a membrane fraction derived from a membrane-bound organell, in particular derived from the cell membrane.
20. The method of claim 17, wherein the Ca^{2+} -binding polypeptide is targeted
10 to the inner surface of the cell membrane.
21. The method of claim 17, wherein step b) is effected by administering an extracellular stimulus, in particular by adding a small chemical compound or a polypeptide to the extracellular side of the host cell.
- 15 22. A method for the detection of the binding of a small chemical coumpound or a polypeptide to a Ca^{2+} -binding polypeptide with an identityof at least 80% to a 30 amino acid long polypeptide sequence of human troponin C or chicken skeletal muscle troponin or drosophila troponin C isoform 1, comprising the following steps:
- 20 a) providing a Ca^{2+} -binding polypeptide according to any one of claims 1 to 12; and
- b) adding a small chemical compound to be tested for binding or a polypeptide to be tested for binding; and
- 25 c) determining the degree of binding by measuring FRET between the donor and the acceptor chromophor of the donor-acceptor-pair of said polypeptide according to any one of claims 1 to 12.
- 30 23. The method of claim 21, wherein the Ca^{2+} -binding polypeptide is derived from human troponin C, and particularly is SEQ ID NO. 4.

24. *Ex vivo* use of a polypeptide according to any one of claims 1 to 12 for the detection of changes in the local Ca^{2+} -concentration close to a cellular membrane.
- 5 25. The use of claim 23, wherein the polypeptide is a polypeptide according to claim 9 and particularly comprises a cell membrane targeting sequence, most preferably a cell membrane targeting sequence mediating localization to the cell membrane of pre- or postsynaptic structures.
- 10 26. Use of a polypeptide according to any one of claims 1 to 12 for the preparation of a diagnostic composition for the detection of changes in the local Ca^{2+} -concentration close to a cellular membrane